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Appraisal of state-of-the-art

Reducing suffering in animal models and procedures involving seizures, convulsions and epilepsy

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ABSTRACT

This report is based on discussions and submissions from an expert working group consisting of veterinarians, animal care staff and scientists with expert knowledge relevant to the field and aims to facilitate the implementation of the Three Rs (replacement, reduction and refinement) in the use of animal models or procedures involving seizures, convulsions and epilepsy. Each of these conditions will be considered, the specific welfare issues discussed, and practical measures to reduce animal use and suffering suggested. The emphasis is on refinement since this has the greatest potential for immediate implementation, and some general issues for refinement are summarised to help achieve this, with more detail provided on a range of specific refinements.

1. Introduction

Animal procedures that can result in seizures, convulsions or epilepsy are used in the development of therapies and treatment regimes for epilepsy[1] and to investigate the underlying pathology of this and related conditions (Löscher, 2011). Seizures, convulsions and epilepsy may also occur as unwanted side effects in other areas of research, for example in models of stroke, or post-operatively in procedures involving neurosurgery. Animal models involving seizures are also used to detect seizure liability in potential new medicines prior to clinical use (Easter et al., 2009).

The procedures used to induce seizures, convulsions or epilepsy, and the conditions themselves, are all recognised to have the potential to cause high levels of suffering in animals and are therefore a priority area for implementing all of the Three Rs (Replacement, Reduction and Refinement; Russell & Burch, 1959). Whilst there are opportunities to replace, reduce and refine these models and procedures, this report will focus predominately on refinement. The potential for refinement will depend upon a number of factors, in particular on the precise scientific question that is being addressed, and on whether or not the adverse effects are a necessary component of the study. If an adverse event cannot be avoided entirely then the approach must be to limit its impact on the animal. Whatever the ultimate requirements of the study, the approach to refinement should always involve careful consideration of what happens to the animal at each step of the study (including: during husbandry and care, scientific procedures and adverse events), and

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[1] The World Health Organisation (2005) currently estimates that 50 million people suffer from epilepsy worldwide. In addition, seizures are a common problem in people who have suffered a stroke, or who have conditions such as autism or Alzheimer's disease. Although some anti-epileptic treatments are available, many have serious side effects and 30–40% of patients suffer from drug-resistant seizures (Stables et al., 2002). It is clear that there is still an unmet medical need for new therapeutic approaches and animal use in this area is likely to continue (Jacobs et al., 2009).

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exploration of the measures that might be taken to avoid or ameliorate any physical or psychological suffering.

The key factors that need to be considered with refinement (and reduction and replacement) opportunities in mind are:

- The purpose of the study
- The translational validity of the model/procedure
- The experimental design, including the statistical power of the study and appropriate literature review
- The nature of the procedures
- The nature and level of severity, including all potential harms to the animals (at all stages of the study)
- The methods used to monitor and record clinical signs of adverse effects
- The expertise of staff performing procedures and monitoring animals.

As an overarching broad principle, the scientific questions should be well defined and studies should then be designed to address these using the fewest number of animals, using the model or procedure with the lowest degree of suffering and utilising well defined humane endpoints.

2. Definitions

Review of the literature shows that the terms 'seizure' and 'convulsion' are often used interchangeably, but it is critical for the purpose of establishing clear guidance for refinement that a distinction is made. The following working definitions apply throughout this report.

Seizures are periods of rhythmic, synchronised abnormal neuronal activity that may result in a number of signs including loss of consciousness, visual disturbance, pain, nausea, headache and may, but not inevitably, lead to convulsions.

Convulsions are a form of generalised seizure characterised by phases of tonic–clonic muscle contractions

Epilepsy is a chronic neurological condition characterised by recurrent epileptic seizures.

The above are induced in animals using similar experimental approaches (Reviewed in: Stables et al., 2002; Löscher, 2011) which are outlined below:

2.1. Electrical-induction

Seizures are induced in animals by passing an electrical current into the brain via electrodes (Racine, 1972a, b; Sato, Racine, & McIntyre, 1990; Wendt, Soerensen, Wotjak, & Potschka, 2011). These can be surgically implanted into a specific brain region (e.g. the limbic system), or applied to the ears (transauricular) or eyes (transcorneal). Electrical shock induction can be used to create a single acute seizure or status epilepticus or be used in a repeated manner starting at a subthreshold level that does not initially provoke seizures (a process known as kindling) to establish a model of epilepsy or status epilepticus.

2.2. Chemical-induction

Animals are given a drug or toxin to induce seizures (Mason & Cooper, 1972; Tetz et al., 2006; Turski et al., 1983). The most common approaches involve the use of pilocarpine, pentylenetetrazole, kainic acid and tetanus toxin (applied to the cortex, not given parenterally). Again, chemical induction can be used to create a single acute seizure (e.g. caffeine, phentylenetetrazole) or to establish a model of status epilepticus, which may lead to the state of recurrent seizures (e.g. pilocarpine, kainic acid, tetanus toxin).

2.3. Mutants and genetically altered animal models

Animals can be used that exhibit spontaneous seizures either in spontaneous mutant or genetically altered (GA) animals (Buchhalter, 1993; Engstrom & Woodbury, 1988; Hosford & Wang, 1997). These include DBA/2, lethargic and stargazer mice and the GAERS (Genetic Absence Epilepsy Rat from Strasbourg) rat (see Section 4.3).

3. General considerations

The scientific question under investigation must be clear from the outset of the study and the model carefully researched and selected so as to maximise translational validity. If the animal use is deemed to be both justified and necessary, the design of the experimental protocol should take into account the potential for suffering of any animals used and how this may be ameliorated as far as possible (including husbandry refinements), whilst still allowing the question to be addressed (Lloyd, Foden, & Wolfensohn, 2008). It is very important to ensure that effective systems are in place for assessing severity and that humane end-points have been clearly defined at the outset in order to minimise suffering. All of these issues are outlined in Sections 3.1 to 3.4 below.

3.1. Translational validity

There is debate in the literature as to the translational validity of some current epilepsy models. There are numerous examples of clinically effective anti-epileptic drugs having no efficacy in some animal models whilst showing benefit in others (Löscher, 2011), suggesting that some current models may have limited predictive capacity for clinical efficacy. There are significant differences between pathologies observed in normal rats subjected to *status epilepticus* and the pattern of pathology in human temporal lobe epilepsy (Mora et al., 2009). Experimental rats rarely exhibit the typical pattern of human hippocampal sclerosis and suffer a much more extensively brain-damaging insult, involving haemorrhage in a multitude of brain structures and ischemic damage (Sloviter, 2005). There needs to be a critical appraisal of the translational validity of animal models of epilepsy and care needs to be taken when selecting the most relevant model for a study in order to ensure that animals are not used inappropriately.

3.2. Experimental design

Rats are the species most commonly used in chronic epilepsy studies. Mice are also used, but are much more prone to variation in response than rats. Care should be taken when selecting the strain of rat for epilepsy models as it has been reported that there are marked strain and substrain differences in the response to both chemical and electrical inductions of epilepsy (Langer, Brandt, & Löscher, 2011). This suggests that data may be highly variable between different research groups and that thorough model validation must be conducted before initiating a new study.

Two major factors determining the level of adverse effects are the number and duration of seizures and/or convulsions that animals experience. Significant refinement can therefore be achieved with experimental designs where both the number and duration of seizures and convulsions are reduced to the minimum necessary. For example, if a novel drug is being tested for seizure liability, then the measurement of a single seizure may be sufficient. Equally, testing the potential antiepileptic activity of a new drug may not need more than a single convulsion. Studies need to be sufficiently powered to permit statistical analysis of the data and to provide confidence that the experimental outcome is correct.

3.3. Severity assessment and humane endpoints

This section outlines general principles with regard to the assessment of severity in models of seizure, convulsions or epilepsy and suggests

some humane endpoints. If reducing animal suffering is to be effectively achieved, suffering must be detected as rapidly as possible so that appropriate action may be taken such as providing analgesia, applying a humane endpoint, reviewing husbandry and enrichment or euthanizing the animal. This may be achieved through effective monitoring of animals and an appreciation of appropriate welfare indicators and can be facilitated by the use of score sheets (Hawkins et al., 2011). An example of a simple score-based approach is given below.

The severity levels of seizures can be classified according to their type (local or generalised), intensity, duration, frequency, the time it takes to recover, and the level of suffering following the incident. The intensity of an induced epileptic seizure can be scored according to the Racine scale (Racine, 1972b):

1. Mouth and facial movement
2. Head nodding
3. Forelimb clonus
4. Rearing with forelimb clonus
5. Rearing and falling with forelimb clonus (generalised motor convulsions).

This scale can form the basis of a more integrated scoring system to help quantify severity in more detail. For example, a scoring system could include: the Racine score, duration of status epilepticus (in hours) and time until normal feeding and drinking resume (in hours). Using this approach, a full *status epilepticus* model of 3 h duration with recovery after 24 h would score highly ($5\times3\times24=360$), whereas a refined model with forelimb clonus for 1 h with recovery after 4 h would score considerably less ($3\times1\times4=12$) and would reflect the more refined model. Additional factors could be included including, but not limited to: weight loss, the side-effects of chemical induction agents (e.g. hypersalivation, diarrhoea), clinical signs of dehydration, duration to normal exploratory activity, return of righting reflex, post seizure behavioural scoring, physical injury due to convulsions and mortality rate.

The intensity, duration and frequency of seizures or convulsions (and their associated side effects) deemed to be acceptable will vary within each project, and there should be agreement between researchers, the local ethical or animal care committee and regulators with respect to humane endpoints on a case by case basis. For example it may be judged that a single seizure of greater than 2 h duration is likely to lead to cortical brain damage and as a result animals would be humanely killed at this point. Similarly, animals may struggle to eat normally following a seizure or convulsion, and if this persists and weight loss of more than 15% of the starting body weight is observed then these animals may also be euthanized.

3.4. Housing and care

Animals experiencing spontaneous seizures or convulsions are likely to have special husbandry needs, and appropriate housing and care should be provided, which may include provisions in addition to usual good practice.[2] For example, although a stimulating environment should be maintained, if there is a risk of injury due to falling it may be necessary to restrict the ability to climb. This could be achieved by just supplying nesting material but not a refuge, or by altering the refuge design so that it cannot be climbed on. The cage floor should always be solid and the type and depth of litter/bedding material also needs to be selected carefully to prevent injury.

Animals may be disorientated following a seizure and this may be ameliorated by returning the animal to a recognisable environment e.g. one with clear visual cues. Individuals may also have difficulty thermoregulating, in which case extra nesting material, litter and/or heated blankets or an incubator may be required (see Gaskill, Rohr, Pajor, Lucas, & Garner, 2009).

Difficulties with normal feeding and drinking may also occur. A modified food source (e.g. moistened chow or liquid nutrition) may be used, but this should be introduced prior to the procedure as some animals are reluctant to try novel food when feeling unwell.

Single housing should be avoided or minimised, or in some circumstances adverse effects of single housing may be mitigated, for example by providing limited (e.g. visual or olfactory) contact. However, this approach may not be beneficial for all species, as a recent study in female C57BL/6JOlaHsd mice showed that for normally socially housed animals, pair housing post surgery with a divider separating the cage mates was more stressful than single housing, presumably because of frustration caused by being able to sense other animals but not fully interact with them (Van Loo et al., 2007). It is important to regularly review the literature on the effects of moving from group to individual housing, including behavioural studies evaluating the effects of limited contact, to ensure that current thinking about good practice is being followed relevant to the particular species under study.

4. Potential for reduction of suffering through refinement of experimental procedures

This section of the report aims to help predict when procedures involving seizures, convulsions or epilepsy are likely to cause suffering and proposes refinements to help avoid or ameliorate this (see Tables 2 to 4). Each component of the animal's experience is considered, with potential measures to help reduce suffering and improve welfare for every stage. This consideration of the 'cumulative effects on the animal' is a useful approach, as sources of pain, suffering or distress are separated out and can be addressed individually (Wolfensohn & Anderson, 2012). This is also in accordance with the approach to severity classification and assessment in some regulations controlling animal use, such as EU Directive 2010/63/EU, and in other guidelines e.g. National Research Council (2008).

Clinically, seizures are reported to occur in three phases, each with associated sensory, emotional and physical symptoms which are summarised in Table 1. Whilst these are symptoms described by patients (material summarised from patient-group websites) it is reasonable to assume that many of these may be experienced by non-human animals during spontaneous or experimentally-induced seizures. For example, patients report memory loss, confusion, depression and fear following a seizure. Since animals are used to study cognition, memory and depression it can be assumed that they are also likely to experience similar sensory and emotional states after seizures (i.e. post-ictal).

4.1. Seizures

It is likely that animals experience little or no suffering *during* a seizure, as consciousness is lost and human patients commonly state that they do not consciously experience or recollect seizures. Human accounts of memory loss are consistent with the involvement of the temporal lobe, the site of short-term memory formation, in seizures (Löscher, 2002). A significant source of suffering is likely to be the procedures (chemical or electrical) that are conducted to induce the seizures, rather than the seizures themselves. For example, peripheral side effects of pilocarpine include salivation, diarrhoea and dehydration, but this can be reduced with a peripherally restricted antimuscarinic such as methylscopolamine (Tetz et al., 2006; Turski et al., 1983). Tetanus toxin can cause paralysis and paresis but this can be avoided with careful administration and with appropriate dose selection. Lithium chloride can be used to reduce the seizure threshold, reduce the required dose of precipitant drug and therefore reduce the incidence and severity of side effects (Terry, Parzernik, & Nelson, 1990). Thus, where seizures are drug-induced, careful consideration of the drug regime and the doses used are fundamental to reducing severity.

[2] http://www.rspca.org.uk/sciencegroup/researchanimals/reportsandresources/housingandcare.

Table 1
Clinical signs of seizure phases in human patients.

Pre-ictal	Ictal	Post-ictal
Sensory/thought:	Sensory/thought:	Sensory/thought:
• Deja vu • Jamais vu • Smells • Sounds • Tastes • Visual loss or blurring • Racing thoughts • Stomach feelings • Tingling feeling	• Black out • Confusion • Deafness/auditory hallucinations • Electric shock feeling • Smell • 'Spacing out' • 'Out of body experience' • Visual loss or blurring	• Memory loss • Impaired function, e.g. writing difficulty
Emotional:	Emotional:	Emotional:
• Fear/panic • Pleasant feeling	• Fear/panic	• Confusion • Depression and sadness • Fear • Frustration
Physical:	Physical:	Physical:
• Dizziness • Headache • Light headedness • Nausea • Numbness	• Breathing difficulty • Convulsion • Drooling • Eyelid fluttering, eyes rolling up • Falling down • Foot stomping, hand waving • Heart racing • Inability to move • Incontinence • Staring • Stiffening • Sweating • Teeth clenching/grinding, tongue biting	• Bruising • Difficulty talking • Injuries • Sleeping • Exhaustion • Headache • Nausea • Pain • Thirst • Weakness • Urge to urinate/ defecate

A significant refinement would therefore be to use a strain of animal that is subject to spontaneous seizures. This may not eliminate suffering completely, because despite remembering little about the seizure event itself, patients often report neurological disturbances (e.g. drowsiness, confusion, nausea, headache or migraine) *before* (pre-ictal) *and after* seizures (as in Table 1) and similar experiences in animals would lead to distress, especially in chronic studies.

Table 2 lists possible adverse events that can lead to seizures, or that may result from experimental paradigms that induce seizures in animals, together with practical refinements.

4.2. Convulsions

Table 3 lists possible adverse events that can result from convulsions, together with practical refinements. Patients do not usually recollect convulsions, but post-convulsion muscle pain and injury are reported and are also likely to apply to animals. This is in conjunction with the potential for psychotic episodes before and after the seizures causing the convulsions, which could be distressing.

Since many, if not all, of the experimental approaches that are used to induce seizures are also used to induce convulsions (with an increase in duration or dose), all of the associated adverse effects and refinements suggested in Table 2 apply as well.

4.3. Epilepsy

Table 4 lists possible adverse effects that can result from experimental paradigms to model epilepsy in animals, with possible refinements. Since many, if not all, of the experimental approaches that are used to induce seizures and convulsions are also used to induce experimental epilepsy (with an increase in duration or dose) all of the refinements suggested in Tables 2 and 3 also apply to epilepsy.

There are many different animal models of epilepsy (Stables et al., 2002). The condition is characterised by spontaneous recurrent seizures and a distinction must be made between (i) models where an episode of *status epilepticus* is induced and (ii) models of epilepsy, where spontaneous recurrent seizures occur. Epilepsy may be induced in animals by drug, toxin or physical damage, or GA lines may be used, either deliberately created or maintained with spontaneous epilepsy (Löscher, 2011).

Surgery and repeated electrical or chemical induction significantly add to the overall level of suffering, so it is good practice to use a strain with spontaneous epilepsy wherever possible to avoid these procedures. An example is the GAERS rat (which has a syndrome similar to absence epilepsy). Note that although the use of GA animals can represent a refinement in that it is not necessary to use regulated procedures to induce epilepsy, there are both ethical and welfare issues associated with the generation and maintenance of GA animal lines. These have been discussed elsewhere (Robinson et al., 2003; Wells et al., 2006).

Chronic models of epilepsy are associated with the highest levels of suffering and mortality. These may involve repeated electrical stimulation (kindling), tetanus toxin injection or, more commonly, subcutaneous or intraperitoneal administration of convulsants (such as kainic acid or pilocarpine).

Experimentally, generation of a model of epilepsy is usually achieved by chemical or electrical induction of *status epilepticus*, which then leads to the development of spontaneous recurrent seizures. These approaches are based on the premise that "seizures beget seizures" (Bertram, 2007) which reflects the clinical phenomenon that for some patients, seizures appear to increase the potential for further seizures.

Traditionally this has involved a single high dose of drug (e.g. pilocarpine) or maximal electric shock (MES), which results in spontaneous recurrent seizures following a 1–2 week quiescent period. This approach is associated with high mortality (up to 80%) and morbidity with weight loss and a high risk of aspiration pneumonia, a pulmonary infection characterised by inflammation and necrosis caused by inhalation of foreign material. Clinical signs include increased laboured breathing, abdominal lift, coughing and raised body temperature. All of these effects are highly undesirable on animal welfare grounds.

However, this approach can be successfully refined by using a seizure threshold lowering agent, such as lithium in conjunction with a lower dose of pilocarpine (Terry et al., 1990). Peripheral side effects of pilocarpine can be ameliorated by using methyl-scopolamine and the initial seizure can be arrested with diazepam. Following a longer quiescent period of 4–10 weeks, spontaneous recurrent seizures are established. This approach results in a much reduced mortality rate of 2% and the data is comparable with the traditional higher dose protocol.

Recently the requirement for induction of *status epilepticus* in order to establish spontaneous recurrent seizures has been challenged (Mora et al., 2009). For example, pilocarpine treated rats have been shown to still experience spontaneous recurrent seizures, after a long latency period, but without developing status epilepticus. This may actually model human frontal lobe epilepsy more closely. It is therefore clear that high-dose models of chemical-induced epilepsy could be replaced with a much more refined low-dose approach, which would greatly reduce animal suffering.

Although analgesia is essential for surgical procedures, there should be no pain associated with seizures. However, whilst it is generally considered that most seizure models do not involve pain, analgesia should be used if there is a reasonable expectation that animals may experience pain as a result of the experimental procedure (for example, following a violent seizure). Analgesic type, dose and route of administration should be selected on the basis of suitability for the species being used, current best practice and, if necessary, a pilot study. Close monitoring of animals for pain-related behaviour is essential (Hawkins, 2002). These

Table 2
Potential adverse events that can lead to or result from seizures and suggested refinements.

Potential adverse effects	How this may be refined
Surgical neurological procedures that may lead to seizures	
Post-operative morbidity	Ensure that the most effective and least aversive anaesthetic agent,
Post-operative pain	that is compatible with the scientific objectives, has been selected
Infection	Provide appropriate perioperative analgesia
Post surgical seizures	Use aseptic technique
Death	Provide antibiotic treatment if necessary
	Ensure that surgical approach is refined so as to minimise tissue damage
	Ensure that surgeon is adequately trained and competent; record
	post-operative outcomes including analgesia requirements
	Review post-operative husbandry and care, including soft diet; heat pads;
	timing of regrouping following surgery; refuge design etc.
	Regularly review post-operative monitoring protocols, including use of score sheets
Surgical implantation of electrodes for kindling	
Post-operative pain	See above
Infection	Use the smallest head-cap possible
Physical impairment/discomfort due to electrode head-cap	Ensure tether system is properly maintained and is suitable for the species
and/or exteriorised electrode leads	Critically review and evaluate the need for single housing. Group house
Behavioural impact of single housing	wherever possible or allow limited contact where it is known to be of benefit
	and will not increase stress
Application of surface electrodes for kindling (e.g. corneal, transauricular)	
Local tissue damage	Minimise duration and intensity of electric shock.
Non-surgical procedures to create seizures, e.g. drug-induced	
Side effects of induction agent (e.g. weakness, excessive salivation,	Use lithium chloride to reduce dose of precipitant required
diarrhoea, dehydration)	Use peripherally selective antagonist to the precipitant to block peripheral
Trauma due to dosing regimen. (e.g. restraint, administration of substances,	side effects (e.g. use methylscopolamine to inhibit peripheral side effects of pilocarpine)
adverse reaction to excipient)	Provide fluid therapy during or post-ictal
	Give wet mash, liquid nutrition, baby food or jelly post-ictal
	Design dosing regimen to limit number and volume of doses and choose an excipient
	that is well tolerated
	Ensure that post-procedure monitoring is effective at picking up clinical signs of
	side effects or trauma
Placing electrodes/telemetry devices to monitor EEG, plus any associated housing constraints	
Post-operative pain	See above
Infection	Consider telemetric EEG devices that transmit data wirelessly, to avoid tethering
Physical impairment due to telemetry device	Use the smallest head-cap possible
Physical impairment/discomfort due to electrode head-cap	If tether system used, ensure that it is properly maintained and is suitable for the species
and/or exteriorised electrode leads.	Ensure that the telemetry device is as small and light as possible, and sited so as to
	minimise impact on the animal
	Question any constraints on husbandry for telemetered and tethered animals,
	e.g. withholding refuges or single housing (Hawkins et al., 2004)
General husbandry	
Behavioural changes leading to anxiety, depression etc.	Refine housing and care so as to improve quality of life, e.g. house social animals
	in stable groups; provide refuges and nesting materials; review cage cleaning
	protocol to minimise disruption
Post-ictal	
Neurological disturbances	Minimise duration of study
	Ensure that animals are closely monitored and use a score sheet to record clinical
	signs if appropriate
	Provide analgesia
	Use of suitable recovery medication such as diazepam
Prolonged seizure >2 h	
Global cortical brain damage	Limit duration of study
	Apply pharmacological intervention to stop seizure
Convulsions	
See below	Reduce seizure-stimulus
	Apply humane end-point (see Table 3)

behaviours may include both obvious signs of discomfort (e.g. hunching, difficulty moving, failure to eat or drink, vocalization, unusually aggressive or passive behaviour, writhing) and more subtle signs (e.g. changes in resting posture, flank twitching, altered respiratory rate). Recently, there have been a series of excellent publications detailing pain-related changes in facial expression in rats, mice and rabbits (Keating, Thomas, Flecknell, & Leach, 2012; Langford et al., 2010; Sotocinal et al., 2011).

5. Opportunities for replacement and reduction

There are a number of possible alternative approaches to investigate seizure mechanisms and the potential seizure liability of new drugs (Easter et al., 2009). These include neuronal–glial cell culture, brain slices, re-aggregated brain homogenates, ion channel affinity assays and *in silico* technology (e.g. neuronal algorithms). In terms of understanding

Table 3
Potential adverse events that can result from convulsions and suggested refinements.

Potential adverse effect	How this may be refined
Disorientation	Refrain from cleaning the recovery cage/environment out for a certain time before or after convulsions for rodents so as not to disrupt olfactory cues that could be used for orientation, provide visual cues. A partial clean may be acceptable as long as sufficient items are left in the cage to provide olfactory cues (Meller et al., 2011) Ensure adequate post-ictal monitoring of the animals
Post-spasm pain	Administer muscle relaxant (e.g. diazepam), either on induction or in the case of repeated convulsions Monitor animals post-ictally for behavioural signs of pain and discomfort and administer analgesics suitable for the species; if post-ictal pain is likely to be a common occurrence, pre-emptive analgesia should be considered
Tooth grinding/ tongue biting	Provide soft foods or liquid nutrition in case it hurts for animals to eat
Injuries from falls	Provide enrichment that animals cannot climb onto, such as refuges with extended walls that prevent animals from climbing onto them; deep soft litter
Swallowing litter or nesting material	Remove if necessary, for the minimum period possible and substitute with a suitable soft cage floor material
Hypothermia if anti-convulsants are used	Provide heat blankets post-ictally Provide plenty of nesting material
Death	Apply humane end-points (e.g. limit to a single convulsion or limit maximum duration of convulsion) Use anti-convulsion treatment (e.g. diazepam or phenobarbitone) to end convulsions Keep detailed records of mortality and associated causes where possible. Review methodology regularly to apply refinements wherever possible

Table 4
Potential adverse events that can result from experimental models of epilepsy and suggested refinements.

Potential adverse effect	How this may be refined
Tissue damage due to repeated electrical shock or repeated drug administration	Use a strain of animals with spontaneous seizures/epilepsy Review administration protocols with respect to properties of substance, doses, routes, magnitude and duration of electrical shock, requirements for handling and restraint
Post-ictal abnormalities e.g. dehydration, electrolyte imbalance, reluctance to eat and drink.	Provide supplementary fluids (i.v. or s.c.) Provide soft foods or liquid nutrition Supply electrolytes in drinking water Test blood chemistry and apply humane end points if electrolyte imbalance cannot be corrected and is likely to result in additional harm to the animal Monitor food consumption and weigh animals regularly. Apply humane end point if weight loss exceeds 15% over three days
Pain	Monitor animals for pain-related behaviour and give appropriate analgesia; if pain is likely to be a common occurrence, pre-emptive analgesia should be considered
Aspiration pneumonia	Reduce number and duration of seizures Use anti-muscarinic drugs to reduce salivation Administer antibiotics appropriate to the species
Permanent damage to brain	Design study to ensure that the induction protocol and number and duration of seizures/convulsions is minimised Ensure adequate monitoring and implement appropriate humane endpoints to limit distressing behavioural and physiological post-ictal symptoms

epilepsy, however, there are currently fewer opportunities for direct replacement. At present, the mechanisms of epileptogenesis are studied using animals, such as rats, with a central nervous system sufficiently complex to show activity similar to that in humans. Some work is conducted on human brain tissue slices from patients who have undergone surgery for intractable epilepsy and multichannel grid recordings can be made from seizure patients during pre-resection monitoring. Whilst these studies yield a relatively small amount of data, opportunities to use human tissue to investigate the fundamental mechanisms that underlie complex conditions such as epilepsy should be taken wherever possible.

Data sharing is a good strategy to reduce and avoid animal use, and the CARMEN (Code Analysis Repository & Modelling for e-Neuroscience; Smith et al., 2007) system for neuroscientists provides opportunities for epilepsy researchers to increase collaboration and share data. CARMEN[3] is a virtual laboratory and data repository that is currently at a pilot stage, with 20 researchers from 11 UK universities involved. This initiative has the potential to be a powerful tool for neuroscientists and, if successful, could form the model for similar initiatives in other research areas. Sharing data and sharing refinement of experimental techniques are critical for wider implementation of the Three Rs, so initiatives such as CARMEN should, if 3Rs data is included, result in higher welfare standards as well as contributing to avoiding animal use.

6. Communicating and disseminating good practice

Dissemination of good practice requires the combined and coordinated efforts of researchers, ethical review committees, journal editors, referees, funders and national professional bodies. This requires a collective understanding of sources of suffering in experiments that use animals, how to identify when these occur and what to do to reduce suffering. Guidance documents from national regulatory authorities as well as from animal welfare groups are useful resources to achieve this, but a collective effort from researchers to report and promote refinement advances is essential if the maximum benefit is to be achieved for animals used in research and testing.

Information relevant to animal welfare should therefore be included in publications arising from work using procedures involving epilepsy, seizures and convulsions. This would increase the implementation of refinement advances and reduce needless repetition of animal studies. There have been a number of recent publications, including the ARRIVE guidelines (Hooijmans, Leenaars, & Ritskes-Hoitinga, 2010; ILAR (Institute for Laboratory Animal Resources), 2011; Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010; Osborne, Phillips, & Westwood, 2010) setting out frameworks for the minimum content that a published article should contain with this regard (these include welfare-related assessments and interventions that were carried out prior to, during, or after the experiment, any adverse events and any implications from the work that may have wider Three Rs benefits).

Researchers collaborating with others who have not been exposed to a well developed culture of animal welfare have a responsibility to disseminate good practice as widely as possible in order to reduce the harms to animals and promote better scientific practice. This can be achieved by sharing detailed protocols, offering training and by emphasising the impact of improved welfare on the quality and relevance of experimental data.

7. Summary

All experimental procedures involving animals should be refined to ensure that suffering has been minimised. Each step of the procedure (including transport, housing, husbandry and care) should be considered, potential adverse effects identified and refinements implemented to

[3] http://www.carmen.org.uk.

ameliorate suffering. Procedures used in the study of seizures, convulsions and epilepsy have the potential for severe suffering in animals. This report gives some practical refinement approaches that can be used to reduce suffering in these procedures and the authors hope that these refinements will be taken, used and further developed by researchers working in this field.

Author's contributions

This manuscript represents the outcome of discussions from an expert working group (EWG) assembled by SW on behalf of the RSPCA. PH, DA, CC, SL, ML, SR, H-M V and GW were all members of the EWG, Norman Flynn (Home Office, UK) was an observer. The manuscript was written by SW, PH and EL and was edited and revised by the EWG.

References

Bertram, E. (2007). The relevance of kindling for human epilepsy. *Epilepsia*, *48*, 65–74.

Buchhalter, J. R. (1993). Animal models of inherited epilepsy. *Epilepsia*, *34*, S31–S41.

National Research Council (2008). *Recognition and alleviation of distress in laboratory animals.* Washington, DC: National Academies Press.

Easter, A., Bell, M. E., Damewood, J. R., Jr., Redfern, W. S., Valentin, J. -P., Winter, M. J., et al. (2009). Approaches to seizure risk assessment in preclinical drug discovery. *Drug Discovery Today*, *14*, 876–884.

Engstrom, F. L., & Woodbury, D. M. (1988). Seizure susceptibility in DBA and C57 mice: The effects of various convulsants. *Epilepsia*, *29*, 389–395.

Gaskill, B. N., Rohr, S. A., Pajor, E. A., Lucas, J. R., & Garner, J. P. (2009). Some like it hot: Mouse temperature preferences in laboratory housing. *Applied Animal Behavioural Science*, *116*, 279–285.

Hawkins, P. (2002). Recognizing and assessing pain, suffering and distress in laboratory animals: A survey of current practice in the UK with recommendations. *Laboratory Animals*, *36*, 378–395.

Hawkins, P., Morton, D. B., Bevan, R., Heath, K., Kirkwood, J., Pearce, P., et al. (2004). Husbandry refinements for rats, mice, dogs and non-human primates used in telemetry procedures. *Laboratory Animals*, *38*, 1–10.

Hawkins, P., Morton, D. B., Burman, O., Dennison, N., Honess, P., Jennings, M., et al. (2011). A guide to defining and implementing protocols for the welfare assessment of laboratory animals: eleventh report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals*, *45*, 1–13.

Hooijmans, C. R., Leenaars, M., & Ritskes-Hoitinga, M. (2010). A gold standard publication checklist to improve the quality of animal studies, to fully integrate the Three Rs, and to make systematic reviews more feasible. *Alternatives To Laboratory Animals*, *38*, 167–182.

Hosford, D. A., & Wang, Y. (1997). Utility of the lethargic (*lh*/*lh*) mouse model of absence seizures in predicting the effects of lamotrigine, vigabatrin, tiagabine, gabapentin, and topiramate against human absence seizures. *Epilepsia*, *38*, 408–414.

ILAR (Institute for Laboratory Animal Resources) (2011). *Guidance for the description of animal research in scientific publications. National Research Council Institute for Laboratory Animal Research.*Washington, DC: The National Academies Press.

Jacobs, M. P., Leblanc, G. G., Brooks-Kayal, A., Jensen, F. E., Lowenstein, D. H., Noebels, J. L., et al. (2009). Curing epilepsy: Progress and future directions. *Epilepsy & Behavior*, *14*, 438–445.

Keating, S. C. J., Thomas, A. A., Flecknell, P. A., & Leach, M. C. (2012). Evaluation of EMLA cream for preventing pain during tattooing of rabbits: Changes in physiological, behavioural and facial expression responses. *PLoS One*, *7*(9), e44437.

Kilkenny, C., Browne, W. J., Cuthill, I. C., Emerson, M., & Altman, D. G. (2010). Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biology*, *8*(6), e1000412.

Langer, M., Brandt, C., & Löscher, W. (2011). Marked strain and substrain differences in induction of status epilepticus and subsequent development of neurodegeneration, epilepsy, and behavioural alterations in rats strain and substrain differences in an epilepsy model in rats. *Epilepsy Research*, *96*, 207–224.

Langford, D. J., Bailey, A. L., Chanda, M. L., Clarke, S. E., Drummond, T. E., Echols, S., et al. (2010). Coding of facial expressions of pain in the laboratory mouse. *Nature Methods*, *7*(6), 447–453.

Lloyd, M. H., Foden, B. W., & Wolfensohn, S. E. (2008). Refinement: Promoting the three Rs in practice. *Laboratory Animals*, *42*, 284–293.

Löscher (2002). Animal models of epilepsy for the development of antiepileptic drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. *Epilepsy Research*, *50*, 105–123.

Löscher (2011). Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. *Seizure*, *20*, 359–368.

Mason, C. R., & Cooper, R. M. (1972). A permanent change in convulsive threshold in normal and brain-damaged rats with repeated small doses of pentylenetetrazol. *Epilepsia*, *13*, 663–674.

Meller, A., Kasanen, I., Ruksenas, O., Apanaviciene, N., Baturaite, Z., Voipio, H. M., et al. (2011). Refining cage change routines: comparison of cardiovascular responses to three different ways of cage change in rats. *Laboratory Animals*, *45*, 167–173.

Mora, G. N., Bramanti, P., Osculati, F., Chakir, A., Nicolato, E., Marzola, P., et al. (2009). Does pilocarpine-induced epilepsy in adult rats require status epilepticus? *PLoS One*, *4*(6), e5759.

Osborne, N., Phillips, B., & Westwood, K. (2010). Journal editorial polices as a driver for change—Animal welfare and the Three Rs. (14–17 June 2010). *Proceedings of the Eleventh FELASA Symposium and the 40th Scand-LAS Symposium*Helsinki, Finland: FELASA http://www.felasa.eu

Racine, R. J. (1972a). Modification of seizure activity by electrical stimulation: I. After-discharge threshold. *EEG and Clinical Neurophysiology*, *32*(3), 269–279.

Racine, R. J. (1972b). Modification of seizure activity by electrical stimulation: II. Motor seizure. *EEG and Clinical Neurophysiology*, *32*(3), 281–294.

Robinson, V., Morton, D. B., Anderson, D., Carver, J. F. A., Francis, R. J., Hubrecht, R., et al. (2003). Refinement and reduction in production of genetically modified mice. *Laboratory Animals*, *37*(Suppl. 1), 1–51.

Russell, W. M. S., & Burch, R. L. (1959). *The principles of humane experimental technique.* London: Methuen.

Sato, M., Racine, R. J., & McIntyre, D. C. (1990). Kindling: Basic mechanisms and clinical validity. *Electroencephalography and Clinical Neurophysiology*, *76*, 459–472.

Sloviter, R. S. (2005). The neurobiology of temporal lobe epilepsy: Too much information, not enough knowledge. *Comptes Rendus Biologies*, *328*, 143–153.

Smith, L. S., Austin, J., Baker, S., Borisyuk, R., Eglen, S., Feng, J., et al. (2007). The CARMEN e-Science pilot project: Neuroinformatics work packages. *Proceedings of the UK e-Science All Hands Meeting*. (pp. 591–598).

Sotocinal, S. G., Sorge, R. E., Zaloum, A., Tuttle, A. H., Martin, L. J., Wieskopf, J. S., et al. (2011). The Rat grimace scale: A partially automated method for quantifying pain in the laboratory rat via facial expressions. *Molecular Pain*, *7*, 55–64.

Stables, J. P., Bertram, E. H., White, H. S., Coulter, D. A., Dichter, M. A., Jacobs, M. P., et al. (2002). Models for epilepsy and epileptogenesis: Report from the NIH workshop, Bethesda, Maryland. *Epilepsia*, *43*(11), 1410–1420.

Terry, J. B., Parzernik, T. L., & Nelson, S. R. (1990). Effect of LiCl pretreatment on cholinomimetic-induced seizures and seizure-induced brain edema in rats. *Neuroscience Letters*, *114*, 123–127.

Tetz, L. M., Rezk, P. E., Ratcliffe, R. H., Gordon, R. K., Steele, K. E., & Nambiar, M. P. (2006). Development of a rat pilocarpine model of seizure/status epilepticus that mimics chemical warfare nerve agent exposure. *Toxicology and Industrial Health*, *22*, 255–266.

Turski, W. A., Cavalheiro, E. A., Schwarz, M., Czuczwar, S. J., Kleinrok, Z., & Turski, L. (1983). Limbic seizures produced by pilocarpine in rats: Behavioural, electroencephalographic and neuropathological study. *Behavioural Brain Research*, *9*, 315–335.

Van Loo, P. L., Kuin, N., Sommer, R., Avsaroglu, H., Pham, T., & Baumans, V. (2007). Impact of 'living apart together' on postoperative recovery of mice compared with social and individual housing. *Laboratory Animals*, *41*, 441–455.

Wells, D. J., Playle, L. C., Enser, W. E., Flecknell, P. A., Gardiner, M. A., Holland, J., et al. (2006). Assessing the welfare of genetically altered mice. *Laboratory Animals*, *40*(2), 111–114.

Wendt, H., Soerensen, J., Wotjak, C. T., & Potschka, H. (2011). Targeting the endocannabinoid system in the amygdale kindling model of temporal lobe epilepsy in mice. *Epilepsia*, *52*, e62–e65.

Wolfensohn, S., & Anderson, D. (2012). *Lifetime experience and cumulative suffering: A report on assessment of lifetime experience and cumulative severity under EU Directive 2010/63.* LASA Forum Summer issue.

World Health Organisation (2005). Atlas: Epilepsy care in the world. http://www.who.int/mental_health/neurology/epilepsy_atlas_introdion.pdf